

HAEMATO-BIOCHEMICAL INDICES OF WHITE BELLED PANGOLIN (*Phataginus tricuspis*) IN CAPTIVITY OF FEDERAL COLLEGE OF WILDLIFE MANAGEMENT, NEW-BUSSA, NIGER STATE, NIGERIA

Fatokun, B.O.^{1*}, Ajayi, S.R.², Adeola A.J.² and Ogialekhe. P.²

¹Department of Animal Health and Production Technology, Federal College of Wildlife Management, Forestry Research Institute of Nigeria, P.M.B. 268, New-Bussa, Niger State, Nigeria.

²Department of Wildlife and Ecotourism Management, Federal College of Wildlife Management, Forestry Research Institute of Nigeria, P.M.B. 268, New-Bussa, Niger State, Nigeria.

Accepted 28, November 2024

Illegal trafficking of the scales of the White bellied pangolin (*Phataginus tricuspis*) for purpose such as its use in traditional medicine in the Asian region has made this species of Wild animals to face the threat of extinction due to. Ex-situ captive breeding and reintroduction programs have been identified to be a key effort in the conservation of the species. The determination of Haemato-biochemical parameters of captive White bellied pangolin (*Phataginus tricuspis*) is vital to assess the physiological status of these animals during health assessment experiments. This work aims at establishing the blood parameters of White bellied pangolin (*Phataginus tricuspis*) in the captivity of Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria and compares the blood parameters with the established blood reference ranges for rescued wild White bellied pangolin (*Phataginus tricuspis*) in New-Bussa and its environs, Niger State, Nigeria. Blood samples collected from eight (8) captive (four males and four female) clinically normal captive White bellied pangolin (*Phataginus tricuspis*) were used to establish the parameters for hematology and serum biochemistry. Potassium level in Male captive White bellied pangolin (*Phataginus tricuspis*) were significantly ($P<0.05$) higher as compared to the potassium levels in the female captive White bellied pangolin (*Phataginus tricuspis*). It was discovered that the levels of the white blood cell count (WBC), neutrophil counts, alanine phosphatase (ALP) and phosphorus in Captive White bellied pangolins were significantly ($P<0.05$) lower when compared with rescued wild White bellied pangolin (*Phataginus tricuspis*). It was also discovered that the levels of the Packed Cell Volume (PCV), Total Protein (TP), globulin and Blood Urea Nitrogen (BUN) were significantly ($P<0.05$) higher in pangolins in captivity when compared with rescued White bellied pangolin (*Phataginus tricuspis*) in New-Bussa, Niger State, Nigeria.

Key words: Haemato-biochemical, Captive, White bellied pangolin, Ex-situ, *Phataginus tricuspis*.

INTRODUCTION

Pangolins are placental mammals belonging to the order *Pholidota* which are characterized by their unique overlapping epidermal scales (Gaudin *et al.*, 2020) which are widely used in traditional medicine in some parts of the world (Aisher, 2016) are composed of flattened, solid and keratinized cells similar to a primate's finger nails. High demand for Pangolin scales for traditional medicine and meat for the luxury of meat market has contributed to increase in the rate of poaching and trafficking of pangolin (Ali *et al.*, 2021). Susceptibility to extinction of this species of animals has been attributed to rapid population decline due to overexploitation and low reproduction rate (Wicker, *et al.*, 2020) and this has necessitated an increasing need for multi-disciplinary conservation strategies that include the integration of *in-situ* (in the wild) and *ex-situ* (in zoological facilities) management processes to save this threatened species (Byers, 2013). Because White bellied pangolins (*Phataginus tricuspis*) had been listed as 'critically endangered species' by the International Union for Conservation of Nature (Challender, 2019). Because of this factor, husbandry and health

programs are important parts of *ex-situ* conservation programs to ensure the captive population remains physiologically healthy optimally (Ali et al., 2021). Haematological parameters of White bellied pangolin (*Phataginus tricuspis*) have been established and conducted for both confiscated and rescued wild White bellied pangolin (*Phataginus tricuspis*) (Ahmad, 2018). However, interpretation and use of reference ranges in the wild need to be done with caution as certain blood parameters may be altered due to the diet and stress of capture or restraining of the animal (Marco, 2000). This is a preliminary work aimed at establishing the haemato-biochemical parameters of captive White bellied pangolins (*Phataginus tricuspis*). This work is aimed at determining the hematology and serum biochemical parameters of captive pangolins in Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria and to determine if there are sex related differences for these parameters. This study also compares the hematology and serum biochemistry of previous work on the rescued *wild White bellied pangolin (Phataginus tricuspis)* in New-Bussa, Niger State, Nigeria. Eight (8) adult captive White bellied pangolin (*Phataginus tricuspis*) of variable ages were transferred from their pen to the Veterinary hospital for routine health checks as recommended by (Ali et al., 2021). The White bellied pangolin (*Phataginus tricuspis*) are kept in individual housing roofed and concreted with earth as the main form of substrate were used in all the enclosures, pine bark is also made available as an added option for the White bellied pangolin (*Phataginus tricuspis*). Climbing structures and nest boxes were also furnished in the enclosure setup. The temperature within the enclosure setup ranges from 24°C to 30°C and a relative humidity between 77 and 98%. They were also fed specially formulated diet consisting of ants, ant eggs, mealworms, insectivore pellets, silkworms, chicken eggs, chitin and calcium powder, and Vitamin B and K, presented in a blended form as described by (Ali et al., 2021). The volume of the housing ranges between 8.88m³ to 42.74 m³ as recommended by Jaffar *et al* (2019).

MATERIALS AND METHODS

Criteria used in the classification of captive Pangolin as described by Ahmad *et al.* (2018) were used in categorizing White bellied pangolin (*Phataginus tricuspis*) as matured and immature animals. White bellied pangolins were considered adult when their body weight was above 4.0 kg in females and above 6.0 kg in males (Ali et al., 2021). The age ranges of the animals in the study were from one year to ten years old. Animals were placed in a gas chamber and anesthetized with 5% isoflurane (Attane, Piramal Healthcare, Bethlehem, PA, USA) with an oxygen flow rate of 3.0 l/min as described by (Ali et al., 2021). Once the animal had been deeply sedated, it was removed from the gas chamber and placed on a mask. Animals were then maintained on 3% isoflurane with flow rate of 1.5 l/min. Health assessments conducted on the anesthetized animals include Physical examination, blood sample collection, radiography and ultrasonography assessment were conducted on the animals. Bloods samples for the determination of haematological and serum biochemical parameters were collected from the coccygeal vein along the ventral midline of the tail as described by (Ali et al., 2021). Prior to disinfection with 70% ethanol, the skin between the scales was cleaned of soil and other debris. A total of 1–3 ml of whole blood was then individually collected separately using a 5 ml syringe (Terumo Co., Tokyo, Japan) with a 21G × 1.5" needle (Terumo Co.). Five hundred microliter (500µl) of blood was then place in MiniCollect® blood collection tubes (Greiner Bio-One, Kremsmünster, Upper Austria, Austria) containing Ethylene-Diamine-Tetraacetic-Acid (EDTA) for hematology and 0.5 to 2.5 ml of blood was placed into plain (without EDTA) blood collection tubes (Greiner Bio-One, Kremsmünster, Upper Austria, Austria) for serum biochemistry studies. The samples were processed within one (1) to two (2) hours from the time of collection. After the health assessment and blood collection, the White bellied pangolin were placed on 100% oxygen at a flow rate of 1.5 l/min for 3 to 5 min. Individual pangolin were subsequently placed in individual pet carriers for recovery. Once each animal had fully recovered from anesthesia, it was returned to the section to be placed back in their pens. The laboratory analysis procedure was carried out based on the procedure described by Ahmad *et al* (2018). The analyses were run on a VetScan HM5 (Abaxis, San Francisco, CA, USA) automated hematology analyzer using the canine setting. Parameters analyzed include Red Blood Cell count (RBC), hemoglobin (Hb) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count (PLT), White Blood Cell Count (WBC), Lymphocytes (LYM), Monocytes (MON), Neutrophils (NEU), Eosinophils (EOS) and Basophils (BASO). Packed cell volume (PCV) was determined by micro-hematocrit centrifugation at 14,000 rpm for 5 min (Hettich Centrifuge Mikro 200, Andreas Hettich GmbH & Co., KG, Tuttlingen, Germany). Blood samples used for serum biochemistry analyses were left to clot for a minimum of 1 hr. Once the blood had clotted, it was centrifuged at 3,750 rpm (4222 MKII, ALC International, Cologno Monzese, Italy) for 5 min to obtain the serum as described by (Ali et al., 2021). All serum biochemical indices analyzed were run with 100 µl of serum using the VetScan 2 analyzer (Abaxis). The serum biochemical indices analyzed includes: Total Protein (TP), Globulin (GLOB), Albumin (ALB), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Amylase (AMY), Total Bilirubin (TBIL), Blood Urea Nitrogen (BUN), Creatinine (CREA), Glucose (GLU), Calcium (Ca), Phosphate (PHOS), Sodium (Na⁺) and Potassium (K⁺). Hematology and serum biochemistry datasets were studied in separate analyses between male and female White Bellied Pangolin as described by (Ali et al., 2021) for Sunda pangolins.

Statistical Analysis

The independent variables gathered were first tested for normality using the Shapiro-Wilks test and Anderson-Darling test as recommended by (Ali et al., 2021) and parametric datasets were then subjected to a Fisher test (F-test) to determine if variance in the data were the same. Two-tailed t-tests were then conducted for datasets that are parametric and for which the variance between the male and female White bellied pangolins were found to be equal. Datasets that were non-parametric were analyzed using the Mann-Whitney U test as described by (Ali et al., 2021). All data statistically analyzed were subjected to 5% level of significance ($P < 0.05$) and descriptive statistics including the mean, median and standard deviation were recorded for the hematological and serum biochemical parameters measured. Minimum and maximum values were presented as the range for the data. The overall dataset for captive White bellied pangolin was then analyzed to determine if it significantly differed from the findings of previous work on rescued adult White bellied pangolins in New-Bussa, Niger State, Nigeria and its environs. The two-tailed t-test was used to test for statistical difference between the normally distributed datasets from both studies. The Mann-Whitney U test was used to test for statistically significant differences between non-parametric datasets from both studies. Cohen D test were conducted on all the dataset to determine the effect of the size of the populations and its relationship between different populations. All analyses were performed using the statistical software R Core Team (2013) version (R Core Team, 2013) as described by (Ali et al., 2021).

RESULTS

A total of eight (8) blood samples (four from males and four from females) were collected from clinically normal adult White bellied pangolin (*Phataginus tricuspis*) were collected after the physiological health assessment. From the results, it was discovered that the level of Potassium were significantly ($P < 0.05$) different between adult male and female White bellied pangolin. Hematological and serum biochemistry variables of the captive adult White bellied pangolin are as seen in Tables 1 and 2.

The comparison between captive and rescued wild White bellied pangolin showed that PCV, TP, globulin and BUN to be significantly ($P < 0.05$) higher in captive White bellied pangolins. On the other hand, WBC counts, neutrophil counts, ALP and phosphorous were found to be significantly ($P < 0.05$) lower in captive White bellied pangolin compared to rescued wild White bellied pangolin. Comparison of the results of the hematological and serum biochemistry between captive and rescued wild White bellied pangolins in New-Bussa and its environs in Niger State, Nigeria can be seen in Tables 3 and 4.

Table 1. Hematological parameters from eight (8) White bellied pangolins (*Phataginus tricuspis*) captivity of Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria.

Parameters	n	Mean	Median	Standard Deviation	Range	Cohen D Test	P value between male and Female
PCV (%) ^a	8	45.20	44.96	4.87	36.00–53.49	0.023	0.9178
Hemoglobin (g/l) ^a	8	149.31	148.82	21.68	116.00–201.00	0.382	0.5191
RBC ($\times 10^{12}/l$) ^a	8	5.63	6.14	1.56	3.02–9.63	0.168	0.7567
MCV (fl) ^a	8	80.28	62.70	4.43	52.50–72.89	0.499	0.3761
MCH (pg) ^b	8	26.52	33.21	7.13	16.64–49.77	0.514	0.7200
MCHC (g/l) ^b	8	330.33	325.00	72.00	62.39–340.01	0.260	0.0735
WBC Count ($\times 10^9/l$)	8	5.01	5.11	1.58	2.17–8.66	0.659	0.2987
Lymphocytes ($\times 10^9/l$) ^a	8	1.01	0.58	0.65	0.19–2.27	0.132	0.8903
Monocytes ($\times 10^9/l$) ^a	8	0.28	0.23	0.22	0.02–0.80	0.471	0.4099
Neutrophils ($\times 10^9/l$) ^a	8	3.63	3.02	1.32	1.31–7.05	0.821	0.1511
Eosinophils ($\times 10^9/l$)	8	0.08	0.03	0.09	0.02–0.37	0.597	0.5092
Basophils ($\times 10^9/l$) ^b	8	0.01	0.01	0.01	0.00–0.04	0.504	0.7812
Platelets ($\times 10^9/l$) ^a	8	139.65	118.97	50.40	75.00–237.00	0.594	0.2055

PCV: packed cell volume; **RBC:** red blood cell count; **MCV:** mean corpuscular volume; **MCH:** mean corpuscular hemoglobin; **MCHC:** mean corpuscular hemoglobin concentration; **WBC Count:** white blood cell count. **a:** Parametric distribution of the data. **b:** Non parametric distribution of the data.

Table 2. Serum biochemical parameters from 8 captive White bellied pangolin (*Phataginus tricuspis*) in captivity of Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria.

Parameters	n	Mean	Median	Standard Deviation	Range	Cohen D Test	P value between male and Female
Total Protein (g/l) ^a	8	78.69	7.93	0.38	6.96 – 8.98	0.2680	0.6047
Globulin (g/l) ^a	8	46.40	45.90	5.19	36.88–56.88	0.9440	0.1033
Albumin (g/l) ^a	8	34.50	34.94	4.66	27.10–45.92	0.9793	0.0678
ALT (U/l) ^b	8	112.40	105.79	75.11	10.00–140.06	0.3572	0.8117
ALP (U/l) ^a	8	218.20	234.54	83.69	104.10 –413.32	0.2181	0.6732
Total Bilirubin (μmol/l) ^a	8	7.91	7.55	1.45	5.91 – 10.79	0.5290	0.1323
Glucose (mmol/l) ^a	8	4.63	4.65	1.10	3.19 – 6.50	0.9540	0.1432
BUN (mmol/l) ^b	8	16.18	11.63	13.68	6.33 – 61.88	0.3183	0.9133
Creatinine (μmol/l) ^a	8	40.49	37.44	24.92	10.54 – 97.00	0.3300	0.5254
Sodium (mmol/l) ^a	8	141.63	140.30	4.33	135.77 – 15.56	0.1112	0.8087
Potassium (mmol/l) ^{a*}	8	3.95	4.20	1.00	2.94 – 6.14	1.4299	0.0230
Combined							
Male	4	4.18	4.45	1.07	4.00 – 6.29		
Female	8	3.68	3.19	0.72	2.82 – 4.15		
Calcium (mmol/l) ^a	8	2.32	2.16	0.16	2.18 – 2.27	0.4165	0.4987
Phosphorous (mmol/l) ^a	8	1.54	1.69	0.32	1.19 – 2.33	0.0823	0.1089
Amylase (U/l) ^a	8	350.90	331.42	94.69	253.00 –590.00	0.3312	0.0873

ALP: alkaline phosphatase; **ALT:** alanine aminotransferase. *The mean between the male and female Sunda pangolins is significantly different (P<0.05). BUN Blood Urea Nitrogen. **a:** Parametric distribution of the data; **b:** Non parametric distribution of the data.

Table 3. Hematological parameters of captive White bellied pangolin (*Phataginus tricuspis*) compared to other published parameters for rescued wild White bellied pangolin in New-Bussa, Niger State, Nigeria and its environs.

Parameters	n	CWBP (Current study) Mean ± SD	n	RWWBP ^b (Previous study) Mean ± SD	Cohen D Test	P values between CWRP
PCV (%) ^a	8	45.93 ± 4.80	16	35.80 ± 6.60	1.2896	0.0063
Hemoglobin (g/l) ^a	8	152.68 ±21.39	16	128.10 ± 18.40	1.2711	0.0610
RBC (×10 ¹² /l)	8	5.98 ± 1.96	16	5.81 ± 1.13	0.8408	0.6300
MCV (fl)	8	65.80 ± 4.60	16	72.66 ± 3.80	0.1397	0.9177
MCH (pg)	8	23.40 ± 8.20	15	24.81 ± 6.80	0.0716	0.2655
MCHC (g/l)	8	304.30 ±72.00	15	301.53 ±144.60	0.1771	0.5810
WBC Count (×10 ⁹ /l) ^a	8	6.12 ± 1.98	16	9.07 ± 3.36	1.2613	0.0016
Lymphocytes (×10 ⁹ /l)	8	1.13 ± 0.70	14	1.42 ± 0.82	0.3313	0.3652
Monocytes (×10 ⁹ /l)	8	0.56 ± 0.25	16	0.74 ± 0.27	0.2699	0.4111
Neutrophils (×10 ⁹ /l) ^a	8	4.21 ± 1.57	15	6.71 ± 3.41	1.0711	0.0023
Eosinophils (×10 ⁹ /l)	8	0.15 ± 0.11	15	.18 ± 0.15	0.2356	0.9648
Basophils (×10 ⁹ /l)	8	0.07 ± 0.02	15	0.02 ± 0.02	0.0000	0.8917
Platelets (×10 ⁹ /l)	8	138.79 ± 48.93	14	116.6 ± 37.80	0.5277	0.1001

CWBP: Captive White bellied pangolins; **RWWBP:** Rescued Wild white bellied pangolins; **CWRP:** Captive and Wild Rescued Pangolin; **PCV:** packed cell volume. **RBC:** red blood cell count; **MCV:** mean corpuscular volume; **MCH:** mean corpuscular hemoglobin; **MCHC:** mean corpuscular hemoglobin concentration; **WBC:** Count, white blood cell count. **a:** Significant differences (P<0.05) were present between the current study on captive White bellied pangolin (*Phataginus tricuspis*) In captivity of Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria and the previous study on rescued wild White bellied pangolin (*Phataginus tricuspis*) in Federal New-Bussa, Niger State, Nigeria and its environs. **b:** Hematology parameters of clinically healthy adult rescued wild White bellied pangolin (*Phataginus tricuspis*) from the previous study.

Table 4. Serum biochemical indices of captive White bellied pangolin (*Phataginus tricuspis*) compared to other published parameters for rescued wild species in New-Bussa, Niger State, Nigeria and its environs.

Parameters	n	CWBP (Current study) Mean \pm SD	n	RWWBP ^b (Previous study) Mean \pm SD	Cohen D Test	P values between CWRP
Total Protein (g/dl) ^b	8	8.53 \pm 0.37	16	7.56 \pm 0.79	1.1237	0.0002
Globulin (g/l) ^b	8	48.50 \pm 6.27	16	37.33 \pm 11.11	0.8523	0.0001
Albumin (g/l)	8	37.50 \pm 4.13	14	38.55 \pm 8.63	0.2476	0.8813
ALT (U/l)	8	114.30 \pm 75.30	15	126.60 \pm 51.50	0.1867	0.3103
ALP (U/l) ^b	8	234.00 \pm 84.90	14	444.30 \pm 172.30	1.5543	0.0001
Total Bilirubin (μ mol/l)	8	8.20 \pm 1.60	16	8.90 \pm 2.80	0.2969	0.2276
Glucose (mmol/l)	8	5.00 \pm 1.20	16	5.50 \pm 1.700	0.3408	0.7234
B U N (mmol/l) ^b	8	17.20 \pm 14.80	15	9.81 \pm 5.18	0.7666	0.0069
Creatinine (μ mol/l)	8	41.60 \pm 25.0	12	34.20 \pm 19.40	0.3419	0.5104
Sodium (mmol/l)	8	143.30 \pm 4.50	16	146.10 \pm 5.30	0.5774	0.4877
Potassium (mmol/l) ^a	8	4.50 \pm 1.10	15	4.60 \pm 0.50	0.7543	0.7113
Calcium (mmol/l)	8	2.58 \pm 0.18	16	2.46 \pm 0.13	0.8776	0.0651
Phosphorous (mmol/l)	8	1.80 \pm 0.40	16	2.30 \pm 0.40	1.2500	0.0002
Amylase (U/l)	8	361.80 \pm 97.90	15	340.0 \pm 49.60	0.280916	0.9063

CWBP: Captive White Bellied Pangolins; **RWWBP:** Rescued Wild White Bellied Pangolins; **CRWWBP:** Captive and Rescued Wild White Bellied Pangolins; **ALP:** alkaline phosphatase; **ALT:** alanine aminotransferase. **a:** Significant differences ($P < 0.05$) were present on the current study on captive White Bellied Pangolin for the analyte between male and female White Bellied Pangolins. **b:** Significant differences ($P < 0.05$) were present between the current study on captive White Bellied Pangolins in Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria and the previous study on rescued wild White Bellied Pangolins in New-Bussa and its environs. **c:** Serum biochemistry parameters of clinically healthy adult rescued wild White Bellied Pangolins from the previous study.

DISCUSSION

In this study we compared the hematology and serum biochemistry dataset between the two studies to determine if there are significant differences between rescued wild and captive White bellied pangolins (*Phataginus tricuspis*). The potassium is significantly higher in the male captive White bellied pangolins compared to the females. Significantly elevated potassium between sexes among healthy individual are infrequently reported. Two studies of wild Canvasback (*Aythya valisineria*) and in human reported significantly higher serum potassium in male but it did not explain the cause of the difference between the sexes in their studies (Wysowski *et al.*, 2003). Further study is required on captive White bellied pangolin (*Phataginus tricuspis*) to explain the significantly higher potassium in male White bellied pangolin. WBC counts, neutrophil counts, phosphorus and ALP in captive White bellied pangolins were found to be significantly lower than those in rescued wild White bellied pangolin. The lower neutrophil and WBC counts of the captive White bellied pangolin could be due to the absence of physiological excitement, which would be present in rescued wild pangolins. Physiological excitement can result in the migration of marginal neutrophils into the bloodstream for circulation. This would lead to the increase of neutrophil and WBC count (Harvey, 2012). As captive pangolins are accustomed to handling of their attendants, it is possible the animals did not experience the same physiological stress as in rescued animals (Ali *et al.*, 2021). The lower phosphorus and ALP level could also be due the absence of physiological excitement in the captive animals as they were brought to the clinic only for anaesthesia and blood sampling. The rescued wild pangolins may have physiological excitement and exertion during its rescue that resulted in elevation of phosphorus (Moen *et al.*, 2010). ALP elevation in rescued wild pangolins could be due to the stress of capture resulting in the release of cortisol. This cortisol will then result in the release of ALP (White *et al.*, 1991). The PCV, TP, globulin and BUN levels of the captive animals were found to be significantly higher compared to that in rescued wild White bellied pangolins. The significantly higher PCV in captive White bellied pangolin could be related to the better nutrition that the animal in the captivity received compared to the free ranging animals (Trumble, 2006). Seasonal increase level of PCV had been recorded in wild western grey kangaroo (*Macropus fuliginosus*) that related to higher availability of protein source of the grazing area (Algar *et al.*, 1988). This study shows globulin levels in captive pangolins to be significantly ($P < 0.05$) higher than in rescued wild White bellied pangolin (*Phataginus tricuspis*). Higher globulin can be indicative of immunological response to an infection or allergy (McGrotty *et al.*, 2016). However, all the White bellied pangolins during the health assessment did not show signs of infection or allergy reaction. Significant differences ($P < 0.05$) were present on the current study on captive White bellied pangolin (*Phataginus tricuspis*) in captivity of Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria for the analyte between male

and female White bellied pangolin (*Phataginus tricuspis*). ^bSignificant differences ($P < 0.05$) were present between the current study on captive White bellied pangolin (*Phataginus tricuspis*) in Wildlife Reserves of the Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria and the previous study on rescued wild White bellied pangolin (*Phataginus tricuspis*) in the experimental site. The lower phosphorus and ALP level could also be due the absence of physiological excitement in the captive animals as they were brought to the hospital for anaesthesia and blood sampling. The rescued wild pangolins may be physiologically excited and exertion during its rescue that resulted in elevation of phosphorus (Moen *et al.*, 2010), (White *et al.*, 1991). ALP elevation in rescued wild pangolins could be due to the stress of capture resulting in the release of cortisol. This cortisol will then result in the release of ALP (White *et al.*, 1991). The PCV, TP, globulin and BUN levels of the captive animals were found to be significantly higher compared to that in rescued wild White bellied pangolin (*Phataginus tricuspis*). The significantly higher PCV in captive White Bellied Pangolins could be related to the better nutrition that the animal in the captivity received compared to the free ranging animals (Hellgren *et al.*, (1989). Trumble *et al.*, (2006). Seasonal increase level of PCV had been recorded in wild western grey kangaroo (*Macropus fuliginosus*) that related to higher availability of protein source of the grazing area (Algar *et al.*, 1988). This study shows globulin levels in captive pangolins to be significantly higher than in rescued wild White bellied pangolin (*Phataginus tricuspis*). Higher globulin can be indicative of immunological response to an infection or allergy (McGrotty *et al.*, 2016). However, all the White bellied pangolin (*Phataginus tricuspis*) during the health assessment did not show signs of infection or allergy reaction. The animals also had no history of infection or allergy prior to the health assessment. The globulin levels in the captive animals could be due to the higher protein diet and better living conditions. These levels could be indicative of increased protein synthesis due to the higher protein diet (Obloh *et al.*, 2008). It is also possible that better nutrition with higher protein assists with building up immunity of the captive animals compare to in the wild (Lander *et al.*, 2003). The significantly higher TP in captive animals were probably related to the significantly higher globulin in captive White bellied pangolin. Elevation of globulin resulted in the increase serum protein concentrate that leads to higher TP value in the captive White bellied pangolin (McGrotty *et al.* 2016). The higher BUN in the captive pangolins could also be due to the higher proportion of protein in the captive White bellied pangolin diet compare to that in the rescued wild or free ranging White bellied pangolin. High protein diets are known to cause elevation in the BUN (Moen *et al.*, 2010). A study published on Asian pangolin captive diet indicates it is similar to the crude protein of wild pangolin diets which consist mainly of invertebrates (Cabana *et al.*, (2017). However, the diet did not factor the high amount of soil and leaf matter consumed by wild pangolins as they feed. The consumption of such matter may dilute the overall protein intake (Lim and Ng 2008). Hence it is possible with the captive diet; pangolins receive higher proportions of crude protein compared to in the wild diet. Establishment of blood parameters for captive animals are important as the hematology and serum biochemistry parameters will be influenced by excitement or stress during the handling or capture of wild animals.

REFERENCES

- Ahmad, A. A., Samsuddin, S., Oh, S. J. W. Y., Martinez-Perez, P. and Rasedee, A. (2018). Hematological and serum biochemical parameters of rescued Sunda pangolins (*Manis javanica*) in Singapore. *Journal of Veterinary Medical Science*, 80(12), 1867-1874.
- Aisher, A. (2016). Scarcity, alterity and value: decline of the pangolin, the world's most trafficked mammal. *Conservation and Society*, 14(4), pp.317-329.
- Algar, D., Arnold, G. W. and Grassia, A. (1988). Effects of nitrogen and season on western grey kangaroo hematology. *The Journal of Wildlife Management*, pp.616-619.
- Ali Anwar Ahmad., Shangari Sekar., Pei yee Oh and Sofeah Samsuddin. (2020). Hematology and serum biochemistry of captive Sunda pangolin (*Manis javanica*) in Wildlife Reserves Singapore. *Journal of Veterinary Medical Science*, 83(2), pp.309-314. doi: 10.1292/jvms.20-0418.
- Byers, O., Lees, C., Wilcken, J. and Schwitzer, C. (2013). The One Plan Approach: The philosophy and implementation of CBSG's approach to integrated species conservation planning. *WAZA magazine* 14: 2–5.
- Cabana, F., Plowman, A., Van Nguyen, T., Chin, S. C., Wu, S. L., Lo, H. Y., Watabe, H. and Yamamoto, F. (2017). Feeding Asian pangolins: An assessment of current diets fed in institutions worldwide. *Zoo Biology*, 36(4), pp.298-305.
- Challender, D., Willcox, D. H. A., Panjang, E., Lim, N., Nash, H., Heinrich, S. and Chong, J. (2019). *Manis javanica*. The IUCN Red List of Threatened species e.T12763A123584856.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T12763A123584856.en [accessed on September 24, 2020].
- Gaudin, T. J., Gaubert, P., Billet, G., Hautier, L., Ferreira-Cardoso, S. and Wible, J. R. (2020). Evolution and morphology. pp. 5–23. In: Pangolins (Challender, D. W. S., Nash, H. C. and Waterman, C. eds.), *Academic Press, London*.
- Harvey, J. W. (2012). Introduction to veterinary hematology. pp. 122–174. In: *Veterinary Hematology: A Diagnostic Guide and Color Atlas, Elsevier Saunders, St. Louis*.
- Hellgren, E. C., Vaughan, M. R. and Kirkpatrick, R. L. (1989). Seasonal patterns in physiology and nutrition of black bears in Great Dismal Swamp, Virginia–North Carolina. *Canadian Journal of Zoology*, 67(8), pp.1837-1850.

- Jaffar, R., Kurniawan, A., Maguire, R., Anwar, A., Cabana, F., Tang, C. and Choo, J. (2019). EAZA Sunda pangolin best practice guidelines, *Wildlife Reserves Singapore Group, Singapore*.
- Lander, M. E., Harvey, J. T. and Gulland, F. M. (2003). Hematology and serum chemistry comparisons between free-ranging and rehabilitated harbor seal (*Phoca vitulina richardsi*) pups. *Journal of Wildlife Diseases*, 39(3), pp.600-609.
- Lim, N. T. and Ng, P. K. (2008). Home range, activity cycle and natal den usage of a female Sunda pangolin *Manis javanica* (*Mammalia: Pholidota*) in Singapore. *Endangered Species Research*, 4(1-2), pp.233-240.
- Marco, I., Martinez, F., Pastor, J. and Lavin, S. (2000). Hematologic and serum chemistry values of the captive European wildcat. *Journal of Wildlife Diseases*, 36(3), pp.445-449.
- McGrotty, Y., Bell, R. and McLauchlan, G. (2016). Disorders of plasma proteins. pp. 123–141. In: *BSAVA Manual of Canine and Feline Clinical Pathology*, 3rd ed. (Villiers, E. and Ristic, J. eds.), British Small Animal Veterinary Association, Gloucester.
- Moen, R., Rasmussen, J. M., Burdett, C. L. and Pelican, K. M. (2010). Hematology, serum chemistry, and body mass of free-ranging and captive Canada lynx in Minnesota. *Journal of Wildlife Diseases*, 46(1), pp.13-22.
- Oboh, H. A. and Olumese, F. E. (2008). Effects of high-protein, low-carbohydrate and fat, Nigerian-like diet on biochemical indices in rabbits. *Pakistan Journal of Nutrition* 7: 640–644.
- Trumble, S. J., Castellini, M. A., Mau, T. L. and Castellini, J. M. (2006). Dietary and seasonal influences on blood chemistry and hematology in captive harbor seals. *Marine Mammal Science*, 22(1), pp.104-123.
- White, P. J., Kreeger, T. J., Seal, U. S. and Tester, J. R. (1991). Pathological responses of red foxes to capture in box traps. *The Journal of Wildlife Management*, pp.75-80.
- Wicker, L. V., Cabana, F., Chin, J. S. C., Jimerson, J., Lo, F. H. Y., Lourens, K., Mohapatra, R. K., Roberts, A. and Wu, S. (2020). Captive husbandry of pangolins: lessons and challenges. pp. 443–459. In: *Pangolins* (Challender, D. W. S., Nash, H. C. and Waterman, C. eds.), *Academic Press, London*.
- Wysowski, D. K., Kornegay, C., Nourjah, P. and Trontell, A. (2003). Sex and age differences in serum potassium in the United States. *Clinical Chemistry*, 49(1), pp.190-192.